

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
25 January 2001 (25.01.2001)

PCT

(10) International Publication Number  
**WO 01/05961 A1**

(51) International Patent Classification<sup>7</sup>: C12N 15/10,  
15/62, 15/70, 15/85, 15/86, 15/63

(72) Inventor: FARMER, Andrew; Apartment 232, 2255 Showers Drive, Mountain View, CA 94040 (US).

(21) International Application Number: PCT/US00/19221

(74) Agent: FIELD, Bret, E.; Bozicevic, Field & Francis LLP, Suite 200, 200 Middlefield Road, Menlo Park, CA 94025 (US).

(22) International Filing Date: 14 July 2000 (14.07.2000)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(25) Filing Language: English

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,

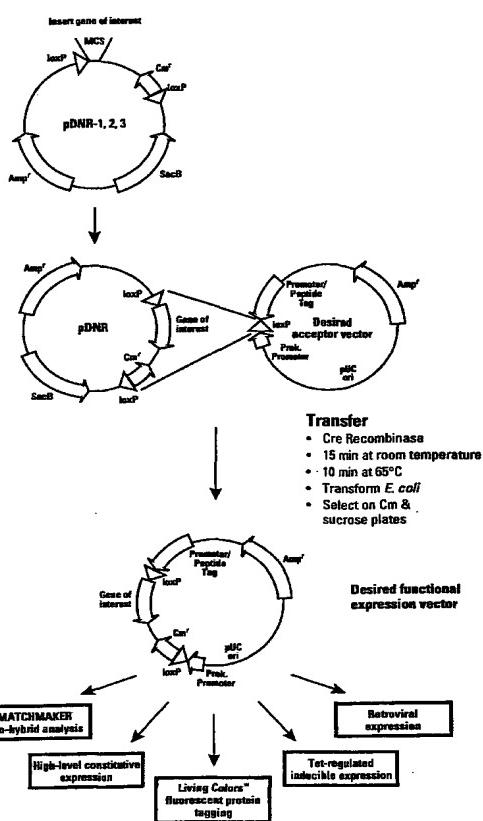
(26) Publication Language: English

(30) Priority Data:  
09/356,001 14 July 1999 (14.07.1999) US

(71) Applicant: CLONTECH LABORATORIES, INC.  
[US/US]; 1020 East Meadow Drive, Palo Alto, CA 94303  
(US).

[Continued on next page]

(54) Title: RECOMBINASE-BASED METHODS FOR PRODUCING EXPRESSION VECTORS AND COMPOSITIONS FOR USE IN PRACTICING THE SAME



(57) Abstract: Methods are provided for producing an expression vector. In the subject methods, donor and acceptor vectors are combined in the presence of a recombinase to produce an expression vector that includes a first and second recombinase recognition site oriented in the same direction, wherein the first and second recombination sites are able to recombine with each other. In the subject methods, one of the donor and acceptor vectors includes a single recombinase recognition site while the other includes two recombinase recognition sites. Also provided are compositions for use in practicing the subject methods, including the donor and acceptor vectors themselves, as well as systems and kits that include the same. The subject invention finds use in a variety of different applications, including the transfer or cloning of a nucleic acid of interest from a first vector into one or more expression vectors, etc.

WO 01/05961 A1



IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,  
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Date of publication of the amended claims:** 28 June 2001

**Published:**

- *With international search report.*
- *With amended claims.*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**AMENDED CLAIMS**

[received by the International Bureau on 24 January 2001 (24.01.01);  
original claim 1-54 replaced by new claims 1-61; (7 pages)]

1. A system for use in preparing an expression vector, said system comprising:  
a donor vector and an acceptor vector, wherein one of said donor and acceptor vectors  
5 comprises two recombinase recognitions sites and the other of said donor and acceptor vectors  
comprises a single recombinase recognition site, wherein all of said recombinase recognition  
sites are able to recombine with each other.
2. The system according to Claim 1, wherein said donor vector comprises two  
10 recombinase recognition sites and said acceptor vector comprises a single recombinase  
recognition site.
3. The system according to Claim 2, wherein said two recombinase recognition sites on  
said donor vector are oriented in the same direction.  
15
4. The system according to Claim 1, wherein said donor vector comprises a single  
recombinase recognition site and said acceptor comprises two recombinase recognition sites.
5. The system according to Claim 4, wherein said two recombinase recognition sites of  
20 said acceptor vector are oriented in the same direction.
6. The system according to Claim 1, wherein said system further comprises a sequence  
specific recombinase.  
25
7. The system according to Claim 6, wherein said sequence specific recombinase is  
selected from the group consisting of: recombinases, transposases and integrases.
8. The system according to Claim 1, wherein said recombinase recognition sites are  
selected from the group consisting of: lox sites, att sites, dif sites and frt sites.  
30
9. The system according to Claim 1, wherein said donor vector and acceptor vector each  
comprise a portion of a selectable marker which are oriented in said vector such that upon  
recombination of said donor and acceptor vectors into an expression vector a functional

selectable marker made up of said donor and acceptor portions is present on said expression vector.

10. The system according to Claim 1, wherein said donor vector comprises two

5 recombinase recognition sites oriented in the same direction and said system further comprises a recombinase.

11. The system according to Claim 1, wherein said donor and acceptor vectors are plasmids, cosmids, bac's, yacs or viruses.

10

12. The system according to Claim 1, wherein said system further comprises a host cell.

13. The system according to Claim 6, wherein said recombinase recognition sites are lox sites and said recombinase is Cre recombinase.

15

14. The system according to Claim 13, wherein said donor vector and acceptor vector each comprise a portion of a selectable marker which are oriented in said vector such that upon recombination of said donor and acceptor vectors into an expression vector a functional selectable marker made up of said donor and acceptor portions is present on said expression vector.

20

15. The system according to Claim 14, wherein said recombinase recognition sites of said donor vector flank a first portion of a selectable marker.

25

16. The system according to Claim 14, wherein single recombinase recognition site of said acceptor vector is located between a first promoter and a second portion of said selectable marker.

30

17. The system according to Claim 16, wherein said second portion of a selectable marker is a second promoter.

18. The system according to Claim 16, wherein said first and second promoters are oriented in opposite directions on said acceptor vector,

19. The system according to Claim 16, wherein said donor and acceptor vectors are plasmids.

20. The system according to Claim 19, wherein said system further comprises a host cell.

5

21. A donor vector comprising:

first and second recombinase recognition sites oriented in the same direction and flanking a portion of a selectable marker, wherein said first and second recombinase recognition sites are able to recombine with each other.

10

22. The donor vector according to Claim 21, wherein said portion of said selectable marker is a coding sequence.

15

23. The donor vector according to Claim 22, wherein said coding sequence is a coding sequence selected from the following group of genes: the chloramphenicol resistance gene, the ampicillin resistance gene, the tetracycline resistance gene, the kanamycin resistance gene, the streptomycin resistance gene and the SacB gene.

20

24. The donor vector according to Claim 21, wherein said recombinase recognition sites are selected from the group consisting of: lox sites, att sites, dif sites and frt sites.

25. The donor vector according to Claim 24, wherein said recombinase recognition sites are lox sites.

25

26. The donor vector according to Claim 21, wherein said donor vector further comprises a second functional selectable marker.

30

27. The donor vector according to Claim 26, wherein said second functional selectable marker is selected from the following group of genes: the chloramphenicol resistance gene, the ampicillin resistance gene, the tetracycline resistance gene, the kanamycin resistance gene, the streptomycin resistance gene and the SacB gene.

28. The donor vector according to Claim 21, wherein said donor vector further comprises a coding sequence for a protein of interest.

29. The donor vector according to Claim 21, wherein said donor vector is a plasmid, cosmid, bac, yac or virus.

30. An acceptor vector comprising:

5 a single recombinase recognition site located between a first promoter and a portion of a selectable marker, wherein said components are positioned such that, upon recombination of said acceptor vector with a donor vector that comprises a coding sequence for a protein of interest flanked by two recombinase recognition sites, an expression vector is produced that comprises an expression cassette made up of said first promoter and said coding sequence

10 which flank a recombinase recognition site.

31. The acceptor vector according to Claim 30, wherein said portion of a selectable marker is a second promoter.

15 32. The acceptor vector according to Claim 31, wherein said second promoter is oriented in the opposite direction of said first promoter.

33. The acceptor vector according to Claim 30, wherein said portion of a selectable marker is a coding sequence for a selectable marker gene.

20 34. The acceptor vector according to Claim 30, wherein said recombinase recognition sites are selected from the group consisting of: lox sites, att sites, dif sites and frt sites.

35. The acceptor vector according to Claim 34, wherein said recombinase recognition site is 25 a lox site.

36. The acceptor vector according to Claim 30, wherein said acceptor vector further comprises an origin of replication.

30 37. The acceptor vector according to Claim 30, wherein said acceptor vector is a plasmid, cosmid, bac, yac or virus.

38. The acceptor vector according to Claim 30, wherein said first promoter is operably linked to a tag encoding sequence.

39. A kit for use in producing an expression vector, said kit comprising a system according to Claim 1.
40. The kit according to Claim 39, wherein said kit further comprises a sequence specific recombinase that recognizes said recombinase recognition sites.
41. A method of producing an expression vector, said method comprising:  
combining a donor vector and an acceptor vector with a recombinase under conditions sufficient for site-specific recombination to occur, wherein one of said donor and acceptor vectors comprises a single recombinase recognition site and the other of said donor and acceptor vectors comprises two recombinase recognition sites and all of said recombinase recognition sites are able to recombine with each other;  
to produce said expression vector that comprises first and second recombinase recognition sites.
42. The method according to Claim 41, wherein said donor vector comprises two recombinase recognition sites and said acceptor vector comprises a single recombinase recognition site.
43. The method according to Claim 42, wherein said donor vector comprises a single recombinase recognition site and said acceptor vector comprises two recombinase recognition sites.
44. The method according to Claim 41, wherein said sequence specific recombinase is selected from the group consisting of: recombinases, transposases and integrases.
45. The method according to Claim 44, wherein said sequence specific recombinase is Cre recombinase.
46. The method according to Claim 41, wherein said recombinase recognition sites are selected from the group consisting of: lox sites, att sites, dif sites and frt sites.
47. The method according to Claim 46, wherein said recombinase recognition sites are lox sites.

48. An expression vector comprising:
- (a) first and second recombinase recognition sites oriented in the same direction;
  - (b) an expression cassette for a protein of interest divided into two subparts that flank said first recombinase recognition site; and
  - 5 (c) a functional marker divided into two sub-parts that flank said second recombinase recognition site.

49. The expression vector according to Claim 48, wherein said recombinase recognition sites are selected from the group consisting of: lox sites, att sites, dif sites and frt sites.

10

50. The expression vector according to Claim 49, wherein said recombinase recognition sites are lox sites.

15

51. The expression vector according to Claim 48, wherein said two subparts of said expression cassette are a promoter and a coding sequence.

52. The expression vector according to Claim 51, wherein said coding sequence of said expression cassette is flanked by said first and second recombinase recognition sites.

20

53. The expression vector according to Claim 48, wherein said two subparts of said selectable marker are a promoter and a coding sequence of a selectable marker.

25

54. The expression vector according to Claim 53, wherein said selectable marker is selected from the following group of genes: the chloramphenicol resistance gene, the ampicillin resistance gene, the tetracycline resistance gene, the kanamycin resistance gene, the streptomycin resistance gene and the SacB gene.

55. The expression vector according to Claim 54, wherein said subparts of said expression cassette are a promoter and a coding sequence, wherein said expression cassette promoter and said selectable marker promoter are oriented in opposite directions.

30

56. The expression vector according to Claim 48, wherein said vector is a plasmid, cosmid, bac, yac or virus..

57. The expression vector according to Claim 56, wherein said vector is a plasmid.

58. A nucleic acid library cloned into a plurality of vectors selected from the group consisting of donor vectors according to Claim 21 and acceptor vectors according to Claim 30.

59. The nucleic acid library according to Claim 58, wherein said library is cloned into a plurality of donor vectors.

60. The nucleic acid library according to Claim 58, wherein said library is a genomic library.

10 61. The nucleic acid library according to Claim 58, wherein said library is a cDNA library.--